Implanting melatonin at lambing enhances lamb growth and maintains high fat content in milk

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Abstract

Three experiments were designed to study the effects of melatonin implantation of ewes and lambs after lambing on the growth of lambs and milk quality throughout lactation. In experiment 1, 53 lambs either did (n = 28) or did not (n = 25) receive a subcutaneous 18-mg melatonin implant. In experiment 2, 55 lambs and their mothers either did (lambs: n = 28; ewes: n = 15) or did not (lambs: n = 27; ewes: n = 16) receive a melatonin implant. Milk samples were collected at 15, 30, and 45 d after lambing. In experiment 3, 16 lambs were separated from their mothers 24 h after birth, moved to an artificial rearing unit, and either did (n = 9) or did not (n = 7) receive a melatonin implant. In the three experiments, implants were inserted 24 h after lambing, and lambs were weighed (LW) weekly until weaning. Average daily gains (ADG) from birth to weaning were calculated. Melatonin treatment of lambs did not have a significant effect on LW at weaning or ADG, but lambs reared by implanted ewes in experiment 2 presented higher ($P < 0.05$) LW (± S.E.M.) at weaning and ADG than did lambs reared by control ewes. At day 45 of lactation, milk fat and total solid content were higher ($P < 0.05$) in implanted ewes than in control ewes. In conclusion, melatonin treatment of ewes at lambing induced a high growth rate of their lambs and increased the fat content of the milk; however, the direct treatment with melatonin of the lambs at birth did not have an effect in their growth rate.

Introduction

Sexual seasonality, which is regulated by photoperiod, limits sheep productivity (Yates 1949). Melatonin is the hormone that transmits the photoperiodic information to the endocrine system, which dictates the precise timing of reproduction. Subcutaneous melatonin implants are a method for artificially controlling oestrus in sheep and, because melatonin is released at night, this procedure is used to cause a short daytime-like response without suppressing endogenous secretion (O'Callaghan et al 1991; Malpaux et al 1997). In Mediterranean genotypes, melatonin implants increased significantly (29%) the probability of pregnancy and fecundity (lambs born/ewe) (0.25 extra lambs/treated ewe) (Palacín et al 2011). Recent studies have shown that, melatonin implants administered between 70 d and 120 d of pregnancy reduced neonatal mortality and high survival rates at weaning, which were mediated through an increase of survival of twins and an increase in tolerance for prolonged parturition in extensively managed sheep flocks (Flinn et al 2020a,b). Melatonin diffuses freely across the ovine placenta and blood–brain barrier (Yellon and Longo 1987; Aly et al 2015), so maternal supplementation is readily able to deliver melatonin to the foetus before birth.

We have observed an improvement in colostrum quality if mothers receive implants at the fourth month of pregnancy (Abecia et al 2020), and lambs reared by implanted ewes were fed more IgG than were lambs from non-implanted ewes. Furthermore, apparently, maternal melatonin in pregnancy plays an important role in the production of brown adipose tissue (BAT) and newborn thermoregulation because melatonin-deficient lambs were colder at birth and reacted abnormally to cold than did control lambs, and the effects of maternal melatonin deficiency were reduced in lambs whose mothers had been kept at a constant photoperiod in pregnancy (Seron-Ferre et al 2015). Probably the improvement of colostrum

quality from implanted ewes and the best thermoregulation of lambs born from melatonin-treated pregnant ewes were responsible for the lower mortality rates and higher growth rates that we documented in Merino lambs reared under extensive conditions in Australia (Davis et al 2021).

This study was designed to quantify the effects of melatonin implantation of ewes and lambs immediately after lambing on the growth of lambs and the quality of milk throughout lactation.

Material And Methods

Experiment 1

The experiment involved 53 Rasa Aragonesa lambs (29 singletons, 24 twins; 29 males, 24 females) born from 40 ewes in the first week of Nov. Lambs either did (group m; n=28; 15 males,13 females) or did not (group c, n=25; 14 males, 11 females) receive an 18-mg melatonin subcutaneous implant (Melovine/Regulin, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear 24 h after birth. Melovine/Regulin is a commercial implant that releases melatonin progressively and at sufficiently concentrations for a period of 70 to 90 days to simulate the shortening of the days (Forcada et al 1999). Positioned at the base of the ear of the animals the implant does not need to be removed, as it is biodegradable.

A digital dynamometer recorded lamb weight at birth and weekly thereafter until weaning (7 weeks). Average daily gain (ADG) from birth to weaning was calculated.

Experiment 2

The experiment involved 55 Rasa Aragonesa lambs (2 singletons, 44 twins, 9 triplets; 31 males, 24 females) born from 31 ewes in the first fortnight of Oct. Twenty-four hours after lambing, ewes either did (group M, n=15) or did not (group C, n=16) receive an 18-mg melatonin subcutaneous implant (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear. At the same time, lambs either did (group m, n=28; 16 males, 12 females) or did not (group c, n=27; 15 males, 12 females) receive a melatonin implant. Thus, lambs were in one of four groups depending on their mother's and their own treatment groups (Cc, n=15; Cm; n=13; Mc, n=12; Mm, n=15). Lambs were weighted at birth and weekly thereafter until weaning (6 weeks). Milk samples were collected at 15 d, 30 d, and 45 d after lambing.

Experiment 3

The experiment involved 16 lambs (8 males, 8 females) born in the first week of Oct, which were separated from their mothers 24 h after birth after having been fed colostrum by their dams. Lambs either did (m, n=9) or did not (c, n=7) receive an 18-mg melatonin subcutaneous implant (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear. They were moved to an artificial rearing unit, which was a 16-m^2 pen that had an electronic milk feeder (Delaval LKF1200). Milk was distributed through a

pipeline system to individual nipples in the pen. Lambs were weighted at birth and weekly thereafter until weaning (5 weeks).

Milk analysis

Milk samples from Experiment 2 were analysed by a milk analyser (Lactoscan SP+) that was calibrated for sheep following the manufacturer's instructions (Milkotronic Ltd., Bulgaria) for measuring fat, protein, lactose, solids not fat (SNF), total solids, and salt contents, and density, freezing point, pH, and conductivity of the milk.

Statistical methods

Experiments 1 and 3 were based on a 2 × 2 factorial design in which the treatment with melatonin of lambs and the sex of the lambs were fixed effects. In experiment 2, a 2 × 2 x 2 factorial design in which the treatment with melatonin of dams and lambs, and the sex of the lambs were fixed effects. The effects of the treatment on lamb growth, ADG, and milk quality (Experiment 2) were evaluated statistically using the GLM PROC (SPSS v. 26) in a model that included melatonin treatment of the lambs, melatonin treatment of their dams (Experiment 2), sex, and their interaction. In Experiment 2, paired-sample t tests for each maternal group were used to detect significant differences in milk quality among days of milk sampling. Results are expressed as mean ± SEM, and the level for statistical significance was set to $p<0.05$.

Results

Experiment 1

Treatment with melatonin or sex of the lambs did not have a significant effect on LW (±S.E.M.) at weaning (m: 14.41 ± 0.42; c: 14.9 ± 0.57 kg) (males: 14.86 ± 0.44; females: 14.4 ± 0.60 kg) or ADG until weaning (m: 221.35 ± 7.80; c: 234.96 ± 10.60 g/d) (males: 230.24 ± 8.35; females: 225.10 ± 10.64 g/d) (Fig. 1).

Experiment 2

*M*elatonin implants in lambs did not have a significant effect on LW at weaning (m: 12.69 ± 0.68; c: 12.87 ± 0.45 kg) or ADG (m: 203.35 ± 0.10; c: 204.60 ± 9.05 g/d) (Table 1); however, lambs reared by melatonin-implanted ewes presented significantly ($P<0.05$) higher LW at weaning (M: 13.61 ± 0.51; C: 12.09 ± 0.57 kg) and ADG (M: 221.00 ± 10.45; 189.92 ± 12.44 g/d) than did lambs from C ewes. Differences between groups in LW were significant from week 3 onwards (Figure 2). The effect of melatonin implantation of the mothers was particularly evident in male lambs (Figure 3); male lambs reared by treated ewes presented significantly higher LW at week 2, 3, and 4 than did male lambs reared by control ewes.

The fat content of milk was significantly ($P<0.05$) higher in M ewes than it was in C ewes at day 45 of lactation, and decreased significantly ($P<0.05$) throughout lactation in C ewes, only (Fig 4). Protein and

lactose content of milk did not differ significantly between groups, although both increased significantly (P<0.05) throughout lactation in the C group. Those differences were apparent in SNF, density, and total solids (P<0.05) in the C group, only, and density in the M group between day 30 and 45. The percentage of total solids was higher (P<0.05) in the M group at day 45 of lactation (Fig 4).

The treatment and control groups did not differ significantly in the pH, conductivity, salt content, and freezing point of the milk, although the conductivity of samples collected on day 30 were significantly (P<0.05) higher than were those collected on day 15 in both groups. The salt content of milk from C ewes increased significantly (P<0.05) throughout lactation, and milk from M ewes increased significantly (P<0.05) from day 15 to day 30. C ewes presented a significant (P<0.05) reduction in the freezing point of their samples from day 30 to day 45.

Experiment 3

Melatonin treatment did not have a significant effect on lamb birth weight (m: 3.61±0.18; c: 3.79±0.28 kg), LW at weaning (m: 10.59±0.85; c: 10.55±0.73 kg), or ADG (m: 0.21±0.01; c: 0.20±0.02 kg/d). The sexes differed significantly in ADG from birth to weaning, but not in LW at birth (males: 3.93±0.24; females: 3.45±0.17 kg) and at weaning (males: 11.37±0.66; females: 9.78±0.87 kg) did not. Females (0.24±0.02 kg/d) had a higher growth rate than did males (0.17±0.02 kg/d) (P<0.05).

Discussion

The main findings of the experiments were the effects of the melatonin treatment of dams at lambing on lamb weight and growth rate, in parallel with an increase in the fat content of the milk, especially at the end of lactation. Furthermore, the milk fat levels of treatment ewes persisted from the beginning to the end of lactation. To our knowledge, this is the first evidence of this effect in a small ruminant, particularly if exogenous melatonin is implanted at lambing. Previous studies on the effects of melatonin implants on milk production and/or offspring growth focused on implantation in the second half of pregnancy or in the middle of the milking period. El Hadi (2020) reported that melatonin implants at day 35 of lactation did not have a significant effect on milk yield and composition in Manchega and Lacaune ewes, and Abecia et al (2005) found that a melatonin implant at mid-milking did not have a significant effect on milk yield throughout the milking period in Lacaune and Assaf dairy ewes. Yang et al (2019) reported a higher fat content in the milk of Cashmere goats that had received an implant at day 50 of lactation than was in the milk of control goats; however, daily yields of milk, milk protein, and milk lactose were lower in the implanted goats than they were in the controls goats. Melatonin administration to does in the dry period, seven weeks before kidding, produced a galactopoietic response in the subsequent lactation and an improved daily weight gain of their suckling kids, especially, males (Avilés et al 2019).

In our experiments, lambs that were reared by implanted ewes had a higher growth rate than did the lambs from control ewes, which might have been due to a higher volume of milk from their implanted dams (not measured), and or the higher fat content of milk, especially at the end of lactation. A high milk fat concentration, milk energy content, and milk energy concentration contributes to faster lamb growth. The administration of exogenous melatonin coupled with the simulation of a short-day photoperiod in

summer had significant effects on the milk levels of solids, protein, fat, and lactose, and on the fatty acid content of sheep milk (Molik et al 2011). Furthermore, the higher content of total solids in the milk of M Rasa Aragonesa ewes might have contributed to the higher growth rates of the lambs because total solids are a combination of fat, protein, lactose, and minerals. In one study, a 30% reduction in overall milk solids was correlated with a 20% reduction in total milk energy production (Muir et al 2000).

Although milk yield was not measured in our experiment, the significant increase in protein and lactose in the control group of ewes throughout lactation, which did not occur in the M group, might indicate lower milk production in the C group because of a dilution effect (Othmane et al 2002). This dilution factor was cited by Bianchi et al (2018) as an explanation for the correlation between low levels of protein and lactose in milk and high milk production in sheep.

The three experiments did not indicate any direct effect of implanting melatonin in the lambs whether they were reared by their dams or artificially. Evidence of treatment with melatonin in suckling lambs is limited. Implanting lambs with a single melatonin sachet subcutaneously on the back at 3–4 weeks of age (Kennaway and Gilmore, 1984) did not affect growth rate, and a pinealectomy of prepubertal sheep did not affect growth rate (Brown and Forbes 1980). Aridas et al (2018) administered melatonin by intravenous infusion or through transdermal patches to newborn lambs that had been subject to induced asphyxia, which reduced the pathologies caused by asphyxia. In newborn lambs, treatment with melatonin reduced the in vivo pulmonary pressor response to changes in oxygenation (Astorga et al 2018) or presented an antiproliferative effect against pathologies such as pulmonary arterial hypertension in neonates (Rivera et al 2020). Melatonin given to human newborns with sepsis reduced the number that died because of its highly effective antioxidant and free-radical scavenging properties (Gitto et al 2001). Although melatonin treatment has positive effects on the newborn’s health, no evidence of an effect on growth rates has been presented. Melatonin treatment of ewes in mid- or late pregnancy has positive effects on lambs. Flinn et al (2020) reported that maternal melatonin supplementation in the second half of pregnancy improved the survival of second-born twin lambs, so that melatonin implants have potential as a simple and cost-effective strategy to reduce neonatal losses of twin lambs on farm. Our group has demonstrated an improvement in colostrum quality if ewes are implanted at the fourth month of pregnancy (Abecia et al 2020), and higher survival and growth rates from birth to weaning of Merino lambs (Davis et al 2021). Probably, the increased survival is mediated by the high colostrum quality, and by an increase in BAT and birth weight if maternal melatonin implants were inserted from day 100 of gestation (Sales et al 2017).

Our experiments indicated no significant differences in the growth rates of male and female lambs, and even among artificially reared lambs, females had higher growth rates than did males. Makovický et al (2019) observed that ewe lambs at the end of milk rearing had a higher average daily gain than did ram lambs, probably, because of differences in the feeding behaviour of males and females fed ad libitum; specifically, females tend to consume less milk per meal than do males, but consume more meals per day than do males (David et al 2014).

The higher growth rate of lambs reared by implanted dams might have been mediated by a higher melatonin concentration in their mother's milk, which in turn might have affected the lamb's health. In cows and goats, milk melatonin levels reflect blood concentrations of melatonin, with a short delay (Eriksson et al 1988), and Cohen et al (2011) speculated that melatonin that is supplied to the infant via breast milk plays a role in improving sleep and reducing colic in breast-fed human offspring (Cohen et al 2011). The lamb can readily produce an appropriately timed melatonin rhythm by 1–6 weeks of age (Claypool et al 1989) and, although maternal melatonin is predominant before birth, significant but low-amplitude increases in nighttime melatonin can occur within the first week (Foster et al 1989). In any case, the absence of differences between c and m lambs from the M group of ewes in Experiment 2, and the results from Experiment 3, in which lambs did not receive milk from their mothers, did not receive extra melatonin from milk, lead us to conclude that neither exogenous or endogenous melatonin of the lambs, or melatonin from their mothers' milk have a direct effect on growth rate.

Conclusions

In conclusion, treatment ewes with melatonin at lambing induced a high growth rate in their lambs and produced an increase in the fat content of the milk; however, the direct treatment with melatonin of the lambs at birth did not have a significant effect on their growth rate.

Declarations

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Authors' contributions Conceptualization, J.A.A. and S.L.; methodology, J.A.A., S.L. and F.C..; investigation, J.A.A. and S.L.; resources, J.A.A.; data curation, S.L.; writing—original draft preparation, J.A.A.; writing—review and editing, J.A.A., S.L. and F.C..; project administration, J.A.A.; funding acquisition, J.A.A. All authors have read and agreed to the published version of the manuscript.

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Compliance with ethical standards The experiment was conducted at the experimental farm of the University of Zaragoza, Spain (41°40'N), under procedures approved by the Ethics Committee for Animal Experiments at the University of Zaragoza, and in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Consent for publication The authors declare consent for publication.

Competing interests The authors declare no competing interests

Availability of data and materials The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

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Table

Table 1 Live weight (±S.E.M.) (LW) at birth and weaning (kg), and average daily gain (ADG) from birth to weaning (kg/d) of Rasa Aragonesa lambs whose mothers either did (M) or did not (C) receive an 18-mg melatonin subcutaneous implant (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear and, at the same time, the lambs either did (m) or did not (c) receive a melatonin implants.

	Cc	Cm	Mc	Mm	Treatment effect	
n	15	13	12	15	lamb	mother
LW Birth	4.23±0.20	3.93±0.13	4.05±0.18	4.23±0.11	ns	ns
LW weaning	12.52±0.56	11.61±1.06	13.40±0.75	13.77±0.74	ns	*
ADG	0.197±0.01	0.182±0.01	0.216±0.01	0.224±0.01	ns	*

ns, non-significant; * P<0.05

Figures

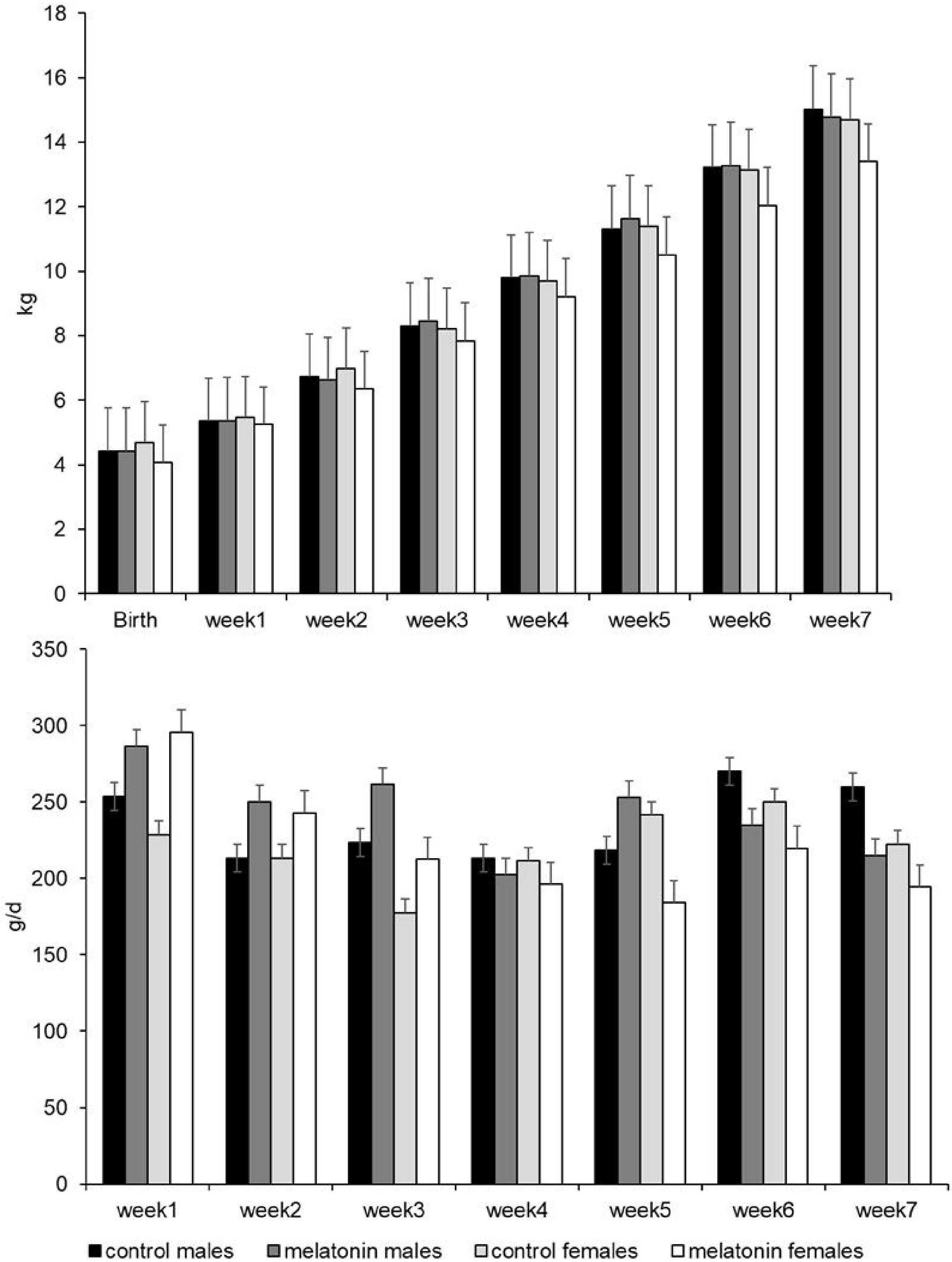

Figure 1

Mean (±S.E.M.) weekly live weight and average daily gain (g/d) of Rasa Aragonesa lambs that either did (n=28; 15 males-13 females) or did not (n=25; 14 males-11 females) receive an 18-mg melatonin subcutaneous implant (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear 24 h after birth.

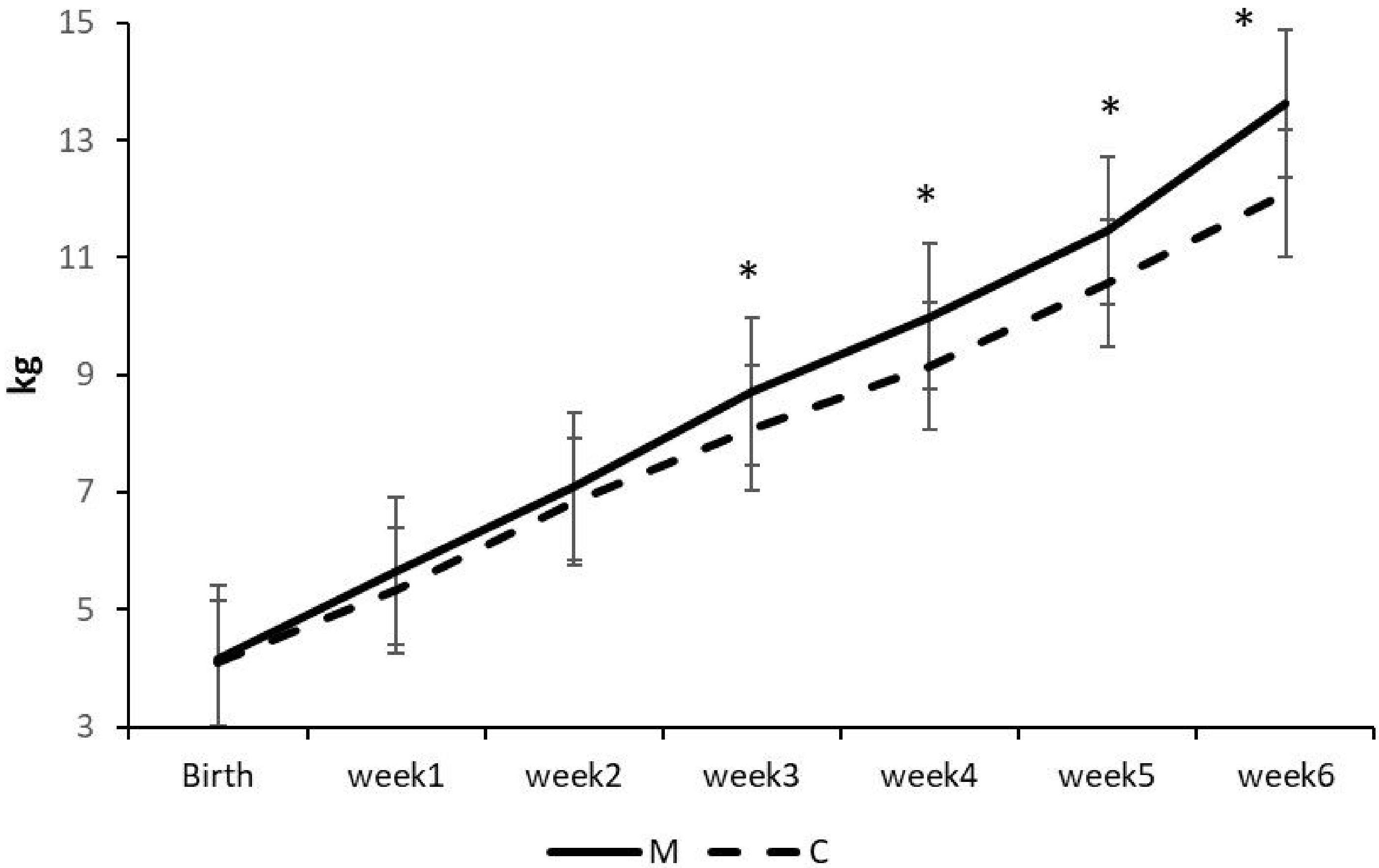

Figure 2

Mean (±S.E.M.) weekly live weight of Rasa Aragonesa lambs whose mothers either did (M) or did not (C) receive an 18-mg melatonin subcutaneous implant (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear 24 h after lambing.

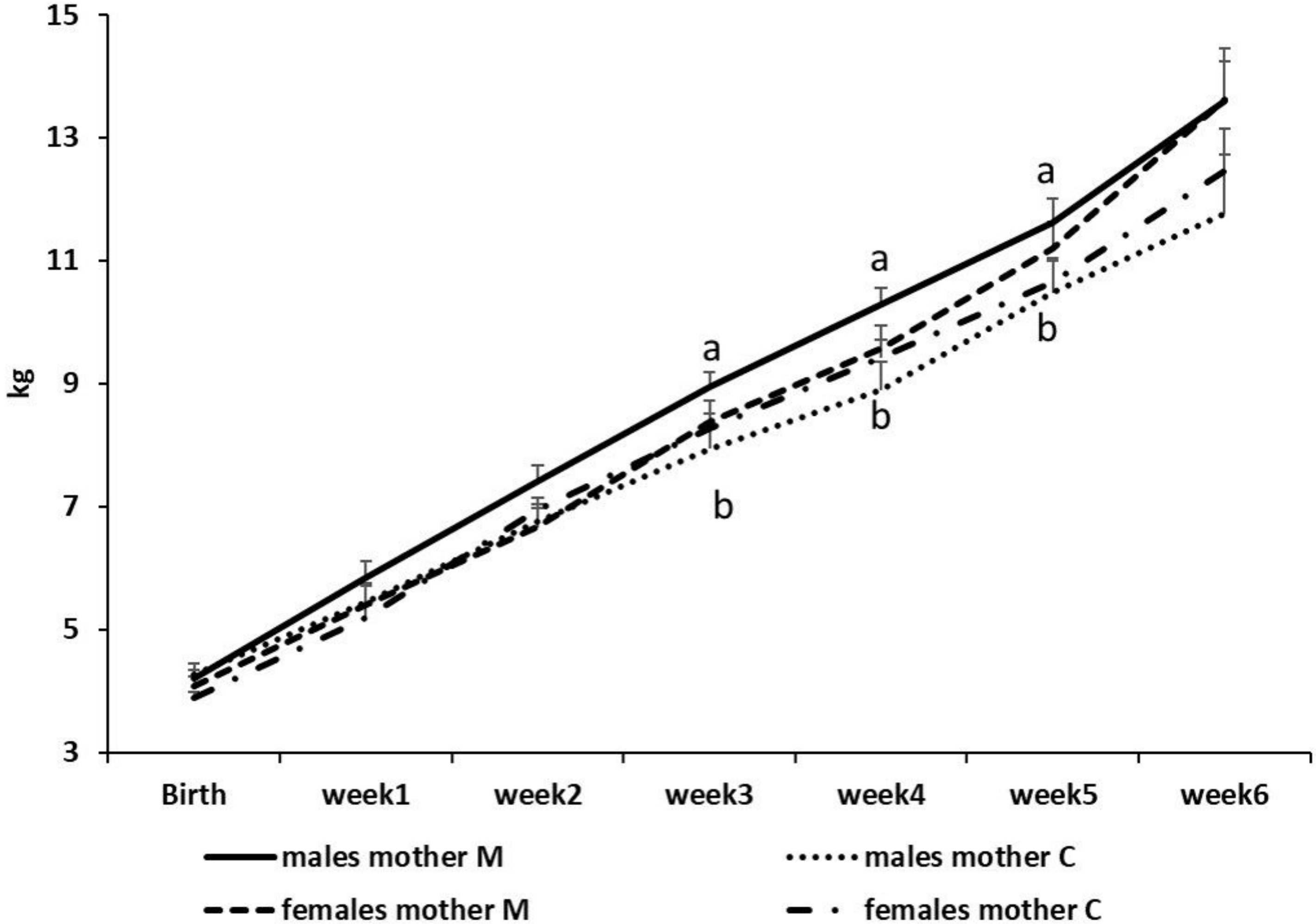

Figure 3

Mean (±S.E.M.) weekly live weight of male and female Rasa Aragonesa lambs whose mothers either did (M) or did not (C) receive an 18-mg melatonin subcutaneous implant (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear 24 h after lambing (letters indicate significant differences at P<0.05).

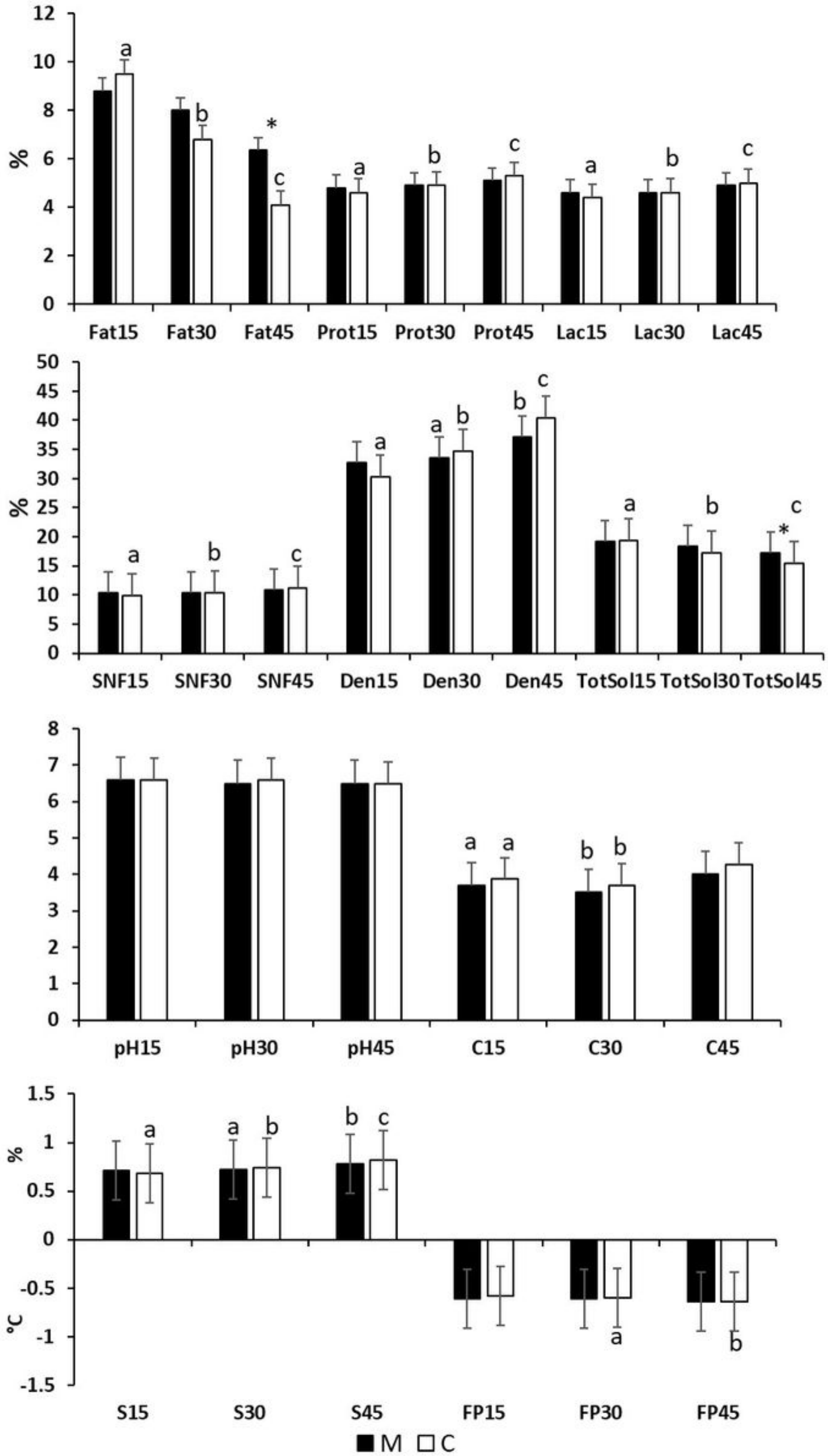

Figure 4

Mean (±S.E.M.) fat, protein (Prot), lactose (Lac), solids not fat (SNF) percentages, and density (Den), total solids (TotSol), pH, conductivity (C), salt content (S), and freezing point (°C) of milk samples collected at days 15, 30, and 45 of lactation from Rasa Aragonesa ewes that either did (M) or did not (C) receive an 18-mg melatonin subcutaneous implant (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of

the left ear 24 h after lambing (letters indicates significant differences at P<0.05 among days; * indicates significant differences between groups at P<0.05).